WEST Search History

DATE: Thursday, August 08, 2002

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT; PLUR=YES; OP=ADJ			
L9	sin-yoke-min.in.	0	L9
L8	gong-zhiyuan.in.	1	L8
L7	L6 and vaccin?$4	4	L7
L6	fish and white spot disease	8	L6
L5	L1 and (i-antigen or immobilization)	2	L5
L4	l1 and recombinant	2	L4
L3	l1 and L2	2	L3
L2	white spot disease	8	L2
L1	ichthyophthirius multifiliis	16	L1

END OF SEARCH HISTORY

9/196161
11/01

```
=> e sin min yoke/au

E1            1     SIN MIN CHOUL/AU
E2            1     SIN MIN CHUOL/AU
E3            0 --> SIN MIN YOKE/AU
E4            1     SIN MING CHIEN/AU
E5            1     SIN MING LAM/AU
E6            1     SIN MINGKUN/AU
E7            1     SIN MUN GIL/AU
E8            1     SIN MYONG HYON/AU
E9            1     SIN MYUNG KYUN/AU
E10          19     SIN N/AU
E11           2     SIN N C/AU
E12           2     SIN N D NIK/AU

=> e min sin yoke/au

E1            1     MIN SIN Y/AU
E2            1     MIN SIN YAKE/AU
E3            3 --> MIN SIN YOKE/AU
E4            1     MIN SIOW W/AU
E5            9     MIN SO YOUN/AU
E6            3     MIN SO YOUNG/AU
E7            1     MIN SOEK R/AU
E8            1     MIN SOHN JUNG/AU
E9            1     MIN SOHN K/AU
E10           3     MIN SOK KI/AU
E11           2     MIN SON HO/AU
E12           1     MIN SON KI/AU

=> s e1-e3

L27           5 ("MIN SIN Y"/AU OR "MIN SIN YAKE"/AU OR "MIN SIN
YOKE"/AU)

=> e jin lam toong/au

E1            2     JIN LAIZHE/AU
E2            1     JIN LAM T/AU
E3            1 --> JIN LAM TOONG/AU
E4           65     JIN LAN/AU
E5            1     JIN LAN D/AU
E6            1     JIN LAN HE/AU
E7            1     JIN LAN L/AU
E8            1     JIN LAN XU SHUHUAI/AU
E9            1     JIN LAN ZHANG SHUYANG/AU
E10           2     JIN LANFEN/AU
E11           1     JIN LANFU/AU
E12           1     JIN LANPING/AU

=> s e2-e3
```

```
L28          2 ("JIN LAM T"/AU OR "JIN LAM TOONG"/AU)

=> e lam toong jin/au

E1           6     LAM TONY Y K/AU
E2           2     LAM TOONG J/AU
E3          20 --> LAM TOONG JIN/AU
E4           4     LAM TRANG/AU
E5           2     LAM TRANG T/AU
E6           1     LAM TRANH H/AU
E7           1     LAM TRI T/AU
E8           1     LAM TRIEU IAN/AU
E9           2     LAM TRIEU LAN/AU
E10          2     LAM TSAN CHIH LI/AU
E11          1     LAM TSANG SING/AU
E12          1     LAM TSE FUN/AU

=> s e2-e3

L29         22 ("LAM TOONG J"/AU OR "LAM TOONG JIN"/AU)

=> e gong zhiyuan/au

E1           1     GONG ZHIYI HUANG YUGUANG/AU
E2           2     GONG ZHIYONG/AU
E3         108 --> GONG ZHIYUAN/AU
E4           1     GONG ZHIYUN/AU
E5          12     GONG ZHIZHONG/AU
E6           1     GONG ZHIZONG/AU
E7           1     GONG ZHODI/AU
E8           2     GONG ZHONG MING/AU
E9           1     GONG ZHONG WEI/AU
E10          3     GONG ZHONGGUI/AU
E11          1     GONG ZHONGHUA/AU
E12          1     GONG ZHONGLIANG/AU

=> s e2-e4

L30        111 ("GONG ZHIYONG"/AU OR "GONG ZHIYUAN"/AU OR "GONG
ZHIYUN"/AU)

=> s l27-l30

   3 FILES SEARCHED...
L31        130 (L27 OR L28 OR L29 OR L30)

=> s l31 and ichthyophthirius

L32          3 L31 AND ICHTHYOPHTHIRIUS

=> d bib ab 1-3


L32  ANSWER 1 OF 3  BIOSIS  COPYRIGHT 2001 BIOSIS
AN   1998:78040  BIOSIS
DN   PREV199800078040
TI   Protection of goldfish against Ichthyophthirius multifiliis by
     immunization with a recombinant vaccine.
AU   He, Jiangyan; Yin, Zhan; Xu, Guoliang; Gond, Zhiyuan; Lam, Toong
```

Jin; Sin, Yoke Min (1)
CS (1) Sch. Biol. Sci., Natl. Univ. Singapore, 10 Kent Ridge Crescent,
Singapore 119260 Singapore
SO Aquaculture, (Dec. 1, 1997) Vol. 158, No. 1-2, pp. 1-10.
ISSN: 0044-8486.
DT Article
LA English
AB A 316 bp gene fragment containing a potential antigenic epitope of the 48
kDa immobilization antigen of **Ichthyophthirius** multifiliis
(i-AgI) was assembled from six synthetic oligonucleotides and cloned into
a bacterial expression vector pGEX2T. The gene construct was introduced
into Escherichia coli and the glutathione S-transferase-iAgI (GST-iAgI)
fusion protein was successfully expressed. Antisera against GST-iAgI
fusion protein from catfish showed a positive reaction with a tomites
protein of about 48 kDa, suggesting that the recombinant protein contains
an antigenic epitope of i-AgI. Naive goldfish that were immunized with
purified GST-iAgI fusion protein were challenged with a lethal dose of
infectious tomites of I. multifiliis. The results showed that the average
survival rate of the immunized fish was 95% as compared to 55% for the
control fish. All these findings suggest that the recombinant GST-iAgI
fusion protein can be used as a potential vaccine against the infection of
I. multifiliis.

L32 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2001 ACS
AN 1997:781004 CAPLUS
DN 128:126832
TI Protection of goldfish against **Ichthyophthirius** multifiliis by
immunization with a recombinant vaccine
AU He, Jiangyan; Yin, Zhan; Xu, Guoliang; **Gong, Zhiyuan**; **Jin Lam, Toong**; **Min Sin, Yoke**
CS 10 Kent Ridge Crescent, School of Biological Sciences, National University
of Singapore, Singapore, 119260, Singapore
SO Aquaculture (1997), 158(1,2), 1-10
CODEN: AQCLAL; ISSN: 0044-8486
PB Elsevier Science B.V.
DT Journal
LA English
AB A 316 bp gene fragment contg. a potential antigenic epitope of the 48 kDa
immobilization antigen of I. multifiliis (i-AgI) was assembled from 6
synthetic oligonucleotides and cloned into a bacterial expression vector
pGEX2T. The gene construct was introduced into Escherichia coli and the

glutathione S-transferase-iAgI (GST-iAgI) fusion protein was successfully expressed. Antisera against GST-iAgI fusion protein from catfish showed a pos. reaction with a tomites protein of about 48 kDa, suggesting that the recombinant protein contains an antigenic epitope of i-AgI. Naive goldfish that were immunized with purified GST-iAgI fusion protein were challenged with a LD of infectious tomites of I. multifiliis. The results showed that the av. survival rate of the immunized fish was 95% as compared to 55% for the control fish. Apparently, the recombinant GST-iAgI fusion protein can be used as a potential vaccine against the infection of I. multifiliis.

L32 ANSWER 3 OF 3 LIFESCI COPYRIGHT 2001 CSA
AN 1998:26672 LIFESCI
TI Protection of goldfish against **Ichthyophthirius** multifiliis by immunization with a recombinant vaccine
AU He, J.; Yin, Z.; Xu, G.; Gong, Z.; **Jin Lam, T.; Min Sin, Y.**
CS School of Biological Sciences, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260, Singapore
SO AQUACULTURE, (19971201) vol. 158, no. 1-2, pp. 1-10. ISSN: 0044-8486.
DT Journal
FS Q4
LA English
SL English
AB A 316 bp gene fragment containing a potential antigenic epitope of the 48 kDa immobilization antigen of **Ichthyophthirius** multifiliis (i-AgI) was assembled from six synthetic oligonucleotides and cloned into a bacterial expression vector pGEX2T. The gene construct was introduced into Escherichia coli and the glutathione S-transferase-iAgI (GST-iAgI) fusion protein was successfully expressed. Antisera against GST-iAgI fusion protein from catfish showed a positive reaction with a tomites protein of about 48 kDa, suggesting that the recombinant protein contains an antigenic epitope of i-AgI. Naive goldfish that were immunized with purified GST-iAgI fusion protein were challenged with a lethal dose of infectious tomites of I. multifiliis. The results showed that the average survival rate of the immunized fish was 95% as compared to 55% for the control fish. All these findings suggest that the recombinant GST-iAgI

fusion protein can be used as a potential vaccine against the infection of
I. multifiliis.

=> s l31 and goldfish

L33 5 L31 AND GOLDFISH

=> d bib ab 1-5

L33 ANSWER 1 OF 5 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1998:78040 BIOSIS
DN PREV199800078040
TI Protection of **goldfish** against Ichthyophthirius multifiliis by immunization with a recombinant vaccine.
AU He, Jiangyan; Yin, Zhan; Xu, Guoliang; Gond, Zhiyuan; **Lam, Toong Jin**; Sin, Yoke Min (1)
CS (1) Sch. Biol. Sci., Natl. Univ. Singapore, 10 Kent Ridge Crescent,
Singapore 119260 Singapore
SO Aquaculture, (Dec. 1, 1997) Vol. 158, No. 1-2, pp. 1-10.
ISSN: 0044-8486.
DT Article
LA English
AB A 316 bp gene fragment containing a potential antigenic epitope of the 48
kDa immobilization antigen of Ichthyophthirius multifiliis (i-AgI) was
assembled from six synthetic oligonucleotides and cloned into a bacterial
expression vector pGEX2T. The gene construct was introduced into
Escherichia coli and the glutathione S-transferase-iAgI (GST-iAgI) fusion
protein was successfully expressed. Antisera against GST-iAgI fusion
protein from catfish showed a positive reaction with a tomites protein of
about 48 kDa, suggesting that the recombinant protein contains an
antigenic epitope of i-AgI. Naive **goldfish** that were immunized
with purified GST-iAgI fusion protein were challenged with a lethal dose
of infectious tomites of I. multifiliis. The results showed that the
average survival rate of the immunized fish was 95% as compared to 55% for
the control fish. All these findings suggest that the recombinant GST-iAgI
fusion protein can be used as a potential vaccine against the infection of
I. multifiliis.

L33 ANSWER 2 OF 5 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1995:264501 BIOSIS
DN PREV199598278801
TI Expression of the antifreeze protein gene in transgenic **goldfish** (Carassius auratus) and its implication in cold adaptation.
AU Wang, Renxue; Zhang, Peijun; **Gong, Zhiyuan**; Hew, Choy L. (1)

CS (1) Dep. Clin. Biochem., Univ. Toronto, 100 College St., Room 351,
Toronto, ON M5G 1L5 Canada
SO Molecular Marine Biology and Biotechnology, (1995) Vol. 4, No. 1, pp.
20-26.
ISSN: 1053-6426.
DT Article
LA English
AB Ocean pout (Macrozoarces americanus) antifreeze protein (AFP) genes (
apprx 10-6 copies) were microinjected into the oocytes of **goldfish**
(Carissius auratus). Out of a total of 303 oocytes injected, 235 (77.6%)
were normally fertilized after in vitro maturation and insemination, and
136 (57.9%) of the fertilized eggs were hatched. Dot blot analysis of
genomic DNA from 2-month-old fish indicated that 30 of the fish (22%) were
positive for the AFP transgene. The numbers of integrated AFP genes ranged
from a single copy to multiple copies per cell. Two P-1 male founders, Y45
and GV16, were crossed separately with a control female, and the
inheritance of the AFP transgene was analyzed. Results from Southern
blotting and polymerase chain reaction were found to be 7% (5 out of 82)
and 58.8% (20 out of 34), respectively, indicating that these two founders
are mosaic. The percentage of positive F-1 generated from Y45F, crossed
with a control female was 56%, consistent with a Mendelian inheritance.
Mature AFP detected by immunoblotting was expressed in both F-1 and F-2
offspring. These studies indicate that the antifreeze protein gene is
successfully transferred and expressed in **goldfish**. Transgenic **goldfish** are significantly more cold tolerant than controls when
challenged with low temperatures. These studies suggest that the AFP gene
may have application in providing cold tolerance in addition to freeze
resistance for a variety of fish species.

L33 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2001 ACS
AN 1997:781004 CAPLUS
DN 128:126832
TI Protection of **goldfish** against Ichthyophthirius multifiliis by immunization with a recombinant vaccine
AU He, Jiangyan; Yin, Zhan; Xu, Guoliang; **Gong, Zhiyuan**; **Jin Lam, Toong**; **Min Sin, Yoke**
CS 10 Kent Ridge Crescent, School of Biological Sciences, National University
of Singapore, Singapore, 119260, Singapore
SO Aquaculture (1997), 158(1,2), 1-10

CODEN: AQCLAL; ISSN: 0044-8486
PB Elsevier Science B.V.
DT Journal
LA English
AB A 316 bp gene fragment contg. a potential antigenic epitope of the 48 kDa immobilization antigen of I. multifiliis (i-AgI) was assembled from 6 synthetic oligonucleotides and cloned into a bacterial expression vector pGEX2T. The gene construct was introduced into Escherichia coli and the glutathione S-transferase-iAgI (GST-iAgI) fusion protein was successfully expressed. Antisera against GST-iAgI fusion protein from catfish showed a pos. reaction with a tomites protein of about 48 kDa, suggesting that the recombinant protein contains an antigenic epitope of i-AgI. Naive **goldfish** that were immunized with purified GST-iAgI fusion protein were challenged with a LD of infectious tomites of I. multifiliis. The results showed that the av. survival rate of the immunized fish was 95% as compared to 55% for the control fish. Apparently, the recombinant GST-iAgI fusion protein can be used as a potential vaccine against the infection of I. multifiliis.

L33 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2001 ACS
AN 1995:686431 CAPLUS
DN 123:80027
TI Expression of the antifreeze protein gene in transgenic **goldfish** (Carassius auratus) and its implication in cold adaptation
AU Wang, Renxue; Zhang, Peijun; **Gong, Zhiyuan**; Hew, Choy L.
CS Inst. Oceanology, Academia Sinica, Shandong, Peop. Rep. China
SO Mol. Mar. Biol. Biotechnol. (1995), 4(1), 20-6
CODEN: MMBBEQ; ISSN: 1053-6426
DT Journal
LA English
AB Ocean pout (Macrozoarces americanus) antifreeze protein (AFP) genes (.apprx.106 copies) were microinjected into the oocytes of **goldfish** (Carassius auratus). Out of 303 oocytes injected, 235 (77.6%) were normally fertilized after in vitro maturation and insemination, and 136 (57.9%) of the fertilized eggs were hatched. Dot blot anal. of genomic DNA from 2-mo-old fish indicated that 30 of the fish (22%) were pos. for the AFP transgene. The nos. of integrated AFP genes ranged from a single copy to multiple copies per cell. Two P1 male founders, Y45 and GV16, were crossed sep. with a control female, and the inheritance of the AFP transgene was analyzed. Results from Southern

blotting and PCR were 7% (5 out of 82) and 58.8% (10 out of 34), resp., indicating that these 2 founders are mosaic. The percentage of pos. F2 generated from Y45F1 crossed with a control female was 56%, consistent with a Mendelian inheritance. Mature AFP detected by immunoblotting was expressed in both F1 and F2 offspring. These studies indicate that the antifreeze protein gene is successfully transferred and expressed in **goldfish**. Transgenic **goldfish** are significantly more cold tolerant than controls when challenged with low temps. These studies suggest that the AFP gene may have application in providing cold tolerance in addn. to freeze resistance for a variety of fish species.

L33 ANSWER 5 OF 5 LIFESCI COPYRIGHT 2001 CSA
AN 1998:26672 LIFESCI
TI Protection of **goldfish** against Ichthyophthirius multifiliis by immunization with a recombinant vaccine
AU He, J.; Yin, Z.; Xu, G.; Gong, Z.; **Jin Lam, T.**; **Min Sin, Y.**
CS School of Biological Sciences, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260, Singapore
SO AQUACULTURE, (19971201) vol. 158, no. 1-2, pp. 1-10. ISSN: 0044-8486.
DT Journal
FS Q4
LA English
SL English
AB A 316 bp gene fragment containing a potential antigenic epitope of the 48 kDa immobilization antigen of Ichthyophthirius multifiliis (i-AgI) was assembled from six synthetic oligonucleotides and cloned into a bacterial expression vector pGEX2T. The gene construct was introduced into Escherichia coli and the glutathione S-transferase-iAgI (GST-iAgI) fusion protein was successfully expressed. Antisera against GST-iAgI fusion protein from catfish showed a positive reaction with a tomites protein of about 48 kDa, suggesting that the recombinant protein contains an antigenic epitope of i-AgI. Naive **goldfish** that were immunized with purified GST-iAgI fusion protein were challenged with a lethal dose of infectious tomites of I. multifiliis. The results showed that the average survival rate of the immunized fish was 95% as compared to 55% for the control fish. All these findings suggest that the recombinant GST-iAgI fusion protein can be used as a potential vaccine against the infection of

I. multifiliis.

=> s l31 and vaccin?

L34 7 L31 AND VACCIN?

=> dup rem l34

PROCESSING COMPLETED FOR L34
L35 3 DUP REM L34 (4 DUPLICATES REMOVED)

=> d bib ab 1-3

L35 ANSWER 1 OF 3 USPATFULL
AN 2001:185451 USPATFULL
TI Intracellular antifreeze polypeptides and nucleic acids
IN Hew, Choy, Thornhill, Canada
Gong, Zhiyuan, Toronto, Canada
PA HSC Research and Development Ltd. Partnership, Toronto, Canada (non-U.S.
corporation)
PI US 6307020 B1 20011023
WO 9728260 19970807
AI US 1998-117121 19981120 (9)
WO 1997-CA62 19970130
19981120 PCT 371 date
19981120 PCT 102(e) date
DT Utility
FS GRANTED
EXNAM Primary Examiner: Carlson, Karen Cochrane; Assistant Examiner: Robinson,
Hope A.
LREP Townsend and Townsend and Crew LLP
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN 21 Drawing Figure(s); 20 Drawing Page(s)
LN.CNT 2175
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A family of related intracellular skin type antifreeze polypeptides and
corresponding coding nucleic acids are provided. These are the first
skin type intracellular antifreeze polypeptides and coding nucleic acids
ever reported. The polypeptides are naturally expressed in the skin of
Winter Flounder, and skin specific promoters are also provided. The
polypeptides are used to make cells cold-resistant, and to improve the
palatability of cold foods and liquids. Cold resistant eukaryotes and
prokaryotes, including plants, animals and bacteria are made using the
skin-type intracellular antifreeze polypeptides and nucleic acids.

L35 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1
AN 2001:334915 BIOSIS
DN PREV200100334915
TI Multiple tissue transformation in adult zebrafish by gene gun bombardment
and muscular injection of naked DNA.
AU Sudha, Puttur Mudumana; Low, Sharon; Kwang, Jimmy; **Gong, Zhiyuan** (1)
CS (1) Department of Biological Sciences, National University of Singapore,
Singapore, 119260: dbsgzy@nus.edu.sg Singapore
SO Marine Biotechnology (New York), (March April, 2001) Vol. 3, No. 2, pp.
119-125. print.
ISSN: 1436-2228.
DT Article
LA English
SL English
AB The efficiency of two direct gene transfer methods, gene gun (or particle
bombardment) and intramuscular injection, in transforming adult zebrafish
tissues in vivo was examined by a noninvasive approach using green
fluorescent protein (GFP) reporter gene driven by the ubiquitously
expressed human cytomegalovirus promoter. Particle bombardment of adult
zebrafish caused internalization and expression of the plasmid only in the
superficial layer such as epithelial cells, pigment cells, endothelial
cells, and neurons, whereas direct injection primarily transformed muscle
fibers of several bundles near or around the injection site. Expression
was also evident in several nonmuscle tissues, such as skin epithelia,
pigment cells, blood vessel cells, and neuron-like cells. GFP expression
persisted for more than 50 days with both methods. These observations
indicate the potential of these methods for functional analysis of
tissue-specific promoters, delivery of DNA **vaccine**, and muscular
expression of other useful genes.

L35 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 2
AN 1998:78040 BIOSIS
DN PREV199800078040
TI Protection of goldfish against Ichthyophthirius multifiliis by
immunization with a recombinant **vaccine**.
AU He, Jiangyan; Yin, Zhan; Xu, Guoliang; Gond, Zhiyuan; **Lam, Toong Jin**; Sin, Yoke Min (1)
CS (1) Sch. Biol. Sci., Natl. Univ. Singapore, 10 Kent Ridge Crescent,
Singapore 119260 Singapore
SO Aquaculture, (Dec. 1, 1997) Vol. 158, No. 1-2, pp. 1-10.
ISSN: 0044-8486.

DT Article
LA English
AB A 316 bp gene fragment containing a potential antigenic epitope of the 48
kDa immobilization antigen of Ichthyophthirius multifiliis (i-AgI) was
assembled from six synthetic oligonucleotides and cloned into a bacterial
expression vector pGEX2T. The gene construct was introduced into
Escherichia coli and the glutathione S-transferase-iAgI (GST-iAgI) fusion
protein was successfully expressed. Antisera against GST-iAgI fusion
protein from catfish showed a positive reaction with a tomites protein of
about 48 kDa, suggesting that the recombinant protein contains an
antigenic epitope of i-AgI. Naive goldfish that were immunized with
purified GST-iAgI fusion protein were challenged with a lethal dose of
infectious tomites of I. multifiliis. The results showed that the average
survival rate of the immunized fish was 95% as compared to 55% for the
control fish. All these findings suggest that the recombinant GST-iAgI
fusion protein can be used as a potential **vaccine** against the
infection of I. multifiliis.

=> d clm 1

L35 ANSWER 1 OF 3 USPATFULL
CLM What is claimed is:
1. An isolated skin-type intracellular antifreeze polypeptide, wherein
the polypeptide comprises an N terminal Met-Asp-Ala-Pro (SEQ ID NO:1)
subsequence; the polypeptide comprises an internal Ala-Ala-Thr-Ala-Ala-
Ala-Ala-Lys-Ala-Ala-Ala (SEQ ID NO:2) subsequence; the polypeptide does
not comprise a signal sequence; the polypeptide induces a
concentration-dependent decrease in the freezing point of an aqueous
solution; and, conservative modifications thereof.

2. The isolated polypeptide of claim 1, wherein the polypeptide has a
molecular of about 3400 Da.

3. The isolated polypeptide of claim 1, wherein the polypeptide has an N
terminal Met-Asp-Ala-Pro-Ala (SEQ ID NO:9) sequence.

4. The isolated polypeptide of claim 1, wherein the polypeptide is from
about 35 to about 55 amino acids in length.

5. The isolated polypeptide of claim 1, wherein the polypeptide comprises the sequence Met-Asp-Ala-Pro-Ala-X.sub.1 -Ala-Ala-Ala-Ala-Thr-
Ala-Ala-Ala-Ala-Lys-Ala-Ala-Ala-Glu-Ala-Thr-X.sub.2 -Ala-Ala-Ala-Ala-
X.sub.2 -Ala-Ala-Ala-X.sub.3 -Thr (SEQ ID NO:3); wherein, X.sub.1 is
selected from the group consisting of Arg, Lys, and Ala; X.sub.2 is
selected from the group consisting of Lys and Ala; and, X.sub.3 is
selected from the group consisting of Ala and Asp and a bond.

6. The isolated polypeptide of claim 1, wherein the polypeptide is
selected from the group consisting of sAFP1 (SEQ ID NO:16), sAFP2 (SEQ
ID NO:18), sAFP3 (SEQ ID NO:20), sAFP4 (SEQ ID NO:22), sAFP5 (SEQ ID
NO:24), sAFP6 (SEQ ID NO:26), sAFP7 (SEQ ID NO:28), sAFP8 (SEQ ID
NO:30), and 11-3 (SEQ ID NO:32).

7. The isolated polypeptide of claim 1, wherein the polypeptide binds t
pool of subtracted polyclonal antibodies, wherein the subtracted
polyclonal antibodies are raised against the sAFP1 (SEQ ID NO:16)
polypeptide and subtracted within HPLC-6 polypeptide (SEQ ID NO:39).

8. The isolated polypeptide of claim 1, wherein the isolated polypeptide
is a component of an aqueous solution.

9. The isolated polypeptide of claim 1, wherein the polypeptide is from
about 60% to about 70% helical as measured by circular dichroism.

10. The isolated polypeptide of claim 1, wherein the polypeptide is a
fusion protein.

11. The isolated skin-type intracellular antifreeze polypeptide of claim
1, which is encoded by a nucleic acid molecule, which nucleic acid
molecule hybridizes to a skin type antifreeze nucleic acid molecule
selected from the group consisting of sAFP1 (SEQ ID NO:15), sAFP2 (SEQ
ID NO:17), sAFP3 (SEQ ID NO:19), sAFP4 (SEQ ID NO:21), sAFP5 (SEQ ID
NO:23), sAFP6 (SEQ ID NO:25), sAFP7 (SEQ ID NO:27), sAFP8 (SEQ ID
NO:29), F2 (SEQ ID NO:33) and 11-3 (SEQ ID NO:31) in a northern blot

under high stringency wash conditions of 0.015M NaCl at 72.degree. C.,
wherein the nucleic acid molecule does not hybridize to SEQ ID NO:41
under high stringency wash conditions of 0.015NaCl at 72.degree. C.

12. The isolated polypeptide of claim 11, wherein the polypeptide is
selected from the group consisting of sAFP1 (SEQ ID NO:16), sAFP2 (SEQ
ID NO:18), sAFP3 (SEQ ID NO:20), sAFP4 (SEQ ID NO:22), sAFP5 (SEQ ID
NO:24), sAFP6 (SEQ ID NO:26), sAFP7 (SEQ ID NO:28), sAFP8 (SEQ ID
NO:30), and 11-3 (SEQ ID NO:32).

13. A method of making an aqueous composition resistant to freezing,
comprising adding a skin-type antifreeze polypeptide to the composition
in an amount sufficient to change the thermal hysteresis of the
composition, wherein the skin-type antifreeze polypeptide comprises an N
terminal Met-Asp-Ala-Pro (SEQ ID NO:1) subsequence, and an internal
Ala-Ala-Thr-Ala-Ala-Ala-Ala-Lys-Ala-Ala-Ala (SEQ ID NO:2) subsequence;
and wherein the polypeptide does not comprise a signal sequence.

14. The method of claim 13, wherein the step of adding the skin type
antifreeze peptide is performed in a cell, wherein the skin type
antifreeze polypeptide is added to the cell by transforming the cell
with a nucleic acid which encodes the skin type antifreeze polypeptide
and expressing the antifreeze polypeptide in the cell.

=> s ichthyophthirius and vaccin?

L36 59 ICHTHYOPHTHIRIUS AND VACCIN?

=> s l36 and (recombin? or DNA or fusion)

L37 25 L36 AND (RECOMBIN? OR DNA OR FUSION)

=> dup rem l37

PROCESSING COMPLETED FOR L37
L38 13 DUP REM L37 (12 DUPLICATES REMOVED)

=> d bib ab 1-13

L38 ANSWER 1 OF 13 USPATFULL
AN 2001:105334 USPATFULL
TI DELIVERY OF NUCLEIC ACID INTO AQUATIC ANIMALS
IN POET, STEVEN E., WINTERVILLE, GA, United States
BURNLEY, VICTORIA VAUGHN, ATHENS, GA, United States
PI US 2001006953 A1 20010705
AI US 1999-347959 A1 19990706 (9)
PRAI US 1998-91820 19980706 (60)
DT Utility
FS APPLICATION
LREP SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.O. BOX 2938, MINNEAPOLIS, MN,
55402
CLMN Number of Claims: 48
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1265
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are methods for delivering a preselected polypeptide into an
aquatic animal by contacting the aquatic animal with an aqueous medium
containing an isolated non-infectious, non-integrating polynucleotide
encoding an immunogen, wherein the polynucleotide is operably linked to
a promoter that controls the expression of the polynucleotide in the
aquatic animal, and wherein expression of the polypeptide stimulates a
detectable biological response in the animal. Also disclosed are methods
for delivering a desired polynucleotide into an aquatic animal
comprising contacting the aquatic animal with an aquatic medium
containing an isolated non-infectious, non-integrating polynucleotide,
wherein the polynucleotide is substantially complementary to all or a
portion of a messenger RNA (mRNA) encoding a preselected polypeptide,
and wherein expression of the polypeptide stimulates or represses a
detectable biological response in the animal. Methods are further
disclosed for delivering a preselected polynucleotide into an aquatic
animal comprising contacting the aquatic animal with an aqueous medium
containing an isolated non-infectious, non-integrating polynucleotide
that is not in contact with a liposome or lipid carrier, wherein the
polynucleotide stimulates a detectable biological response in the
animal.

L38 ANSWER 2 OF 13 USPATFULL
AN 2001:55947 USPATFULL
TI Methods and products for stimulating the immune system using

immunotherapeutic oligonucleotides and cytokines
IN Krieg, Arthur M., Iowa City, IA, United States
Weiner, George, Iowa City, IA, United States
PA University of Iowa Research Foundation, Iowa City, IA, United States
(U.S. corporation)
PI US 6218371 B1 20010417
AI US 1999-286098 19990402 (9)
PRAI US 1998-80729 19980403 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Yucel, Remy; Assistant Examiner: Zara, Jane
LREP Wolf, Greenfield & Sacks, P.C.
CLMN Number of Claims: 23
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 2746
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to synergistic combinations of immunostimulatory CpG oligonucleotides and immunopotentiating cytokines.
In particular, the invention relates to methods of stimulating an immune
response using the synergistic combination of compounds and products
related thereto.

L38 ANSWER 3 OF 13 USPATFULL
AN 2001:14470 USPATFULL
TI **DNA** based **vaccination** of fish
IN Davis, Heather L., Ottawa, Canada
PA Loeb Health Research Institute at The Ottawa Hospital, Ottawa, Canada
(non-U.S. corporation)
PI US 6180614 B1 20010130
AI US 1998-115423 19980714 (9)
RLI Continuation of Ser. No. US 1996-740805, filed on 4 Nov 1996, now
patented, Pat. No. US 5780448, issued on 14 Jul 1998
PRAI US 1995-6290 19951107 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Salimi, Ali R.
LREP Yankwich, Leon R., O'Obrien, David G.
CLMN Number of Claims: 84
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1280
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to methods of immunization of aquaculture
species by introducing **DNA** expression systems into the aquaculture species. Such **DNA** expression systems preferably include **DNA** sequences encoding polypeptides of pathogens of species of aquaculture. The present invention also relates to methods of
administration of **DNA** expression systems into aquaculture.
Such methods include injection, spray, and immersion techniques. The

methods of this invention are useful for prophylactic **vaccination** or therapeutic immunization of fin-fish, shellfish, or other aquatic animals against infectious diseases.

L38 ANSWER 4 OF 13 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 1
AN 2001299812 EMBASE
TI Host responses against the fish parasitizing ciliate **Ichthyophthirius** multifiliis.
AU Buchmann K.; Sigh J.; Nielsen C.V.; Dalgaard M.
CS K. Buchmann, Dept. of Veterinary Microbiology, Section of Fish Diseases,
Royal Veterinary and Agric. Univ., 4 Stigbojlen, DK-1870 Frederiksberg C,
Denmark. kurt.buchmann@vetmi.kvl.dk
SO Veterinary Parasitology, (12 Sep 2001) 100/1-2 (105-116).
Refs: 71
ISSN: 0304-4017 CODEN: VPARDI
PUI S 0304-4017(01)00487-3
CY Netherlands
DT Journal; Conference Article
FS 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English
AB Recent studies have shown that fish are able to mount protective immune
responses against various parasites. One of the best characterized
parasite-host system in this context is the ciliate **Ichthyophthirius** multifiliis (Ich) parasitizing a range of freshwater fishes. Both specific and non-specific host defence mechanisms
are responsible for the protection of fish against challenge infections
with this ciliate. The specific humoral components comprise at least
specific antibodies. The non-specific humoral elements included are the
alternative complement pathway and probably lectins. Cellular factors
involved in the specific response are B-cells and putative T-cells. The
non-specific effector cells recognized are various leukocytes. In addition, goblet-cells and mast cells (EGC-cells) may have a function. The
NCC-cell (suggested analogue to NK-cells in mammals) seems to play a role
in the non-specific response. This well documented protective response in
freshwater fishes against Ich has urged the development of anti-parasitic
vaccines. Indeed, such products based on formalin killed parasites
have been developed and found to offer the **vaccinated** host a satisfactory protection. However, the collection of parasites for **vaccine** production is extremely laborious. It involves keeping infected fish due to the fact that in vitro propagation of the parasite is

still insufficiently developed. Gaining knowledge of amino acid sequences and its encoding **DNA**-sequences for the protective antigens (i-antigens) in the parasite was a major breakthrough. That achievement made it possible to produce a **recombinant** protein in E. coli and preliminary results indicated a certain protection of fish **vaccinated** with this product. Recent work has shown that the free-living and easily cultivated ciliate Tetrahymena can be transformed and express the i-antigen. This path seems to be promising for future development of **vaccines** against Ich. A novel approach in fish is the development of **DNA-vaccines**. Successful **DNA-vaccination** trials have been conducted in fish against viral infections and the technology also makes it possible to develop a **DNA-vaccine** against Ich. Other approaches to immuno-protection against Ich have been the use of heterologous **vaccines**. Thus, both bath and injection **vaccination** using live or killed (un-transformed) Tetrahymena has been reported to offer treated fish a certain level of protection. Such protection could be explained by non-specific reactions and the efficacy and duration of this **vaccination** type should be further evaluated. .COPYRGT. 2001 Elsevier Science B.V. All rights reserved.

L38 ANSWER 5 OF 13 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD DUPLICATE 2

AN 2000-514962 [46] WPIDS

CR 2000-506071 [44]

DNN N2000-380572 DNC C2000-153683

TI **Recombinant** expression systems for expressing heterologous nucleic acids and producing **recombinant** protein, comprises nonpathogenic protozoa such as Tetrahymena resistant to paclitaxel.

DC B04 C06 D16 S03

IN CLARK, T G; DICKERSON, H W; GAERTIG, J

PA (CLAR-I) CLARK T G; (DICK-I) DICKERSON H W; (GAER-I) GAERTIG J; (UYGE-N) UNIV GEORGIA RES FOUND INC

CYC 89

PI WO 2000046381 A1 20000810 (200046)* EN 83p

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW

W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

AU 2000027563 A 20000825 (200059)

ADT WO 2000046381 A1 WO 2000-US2966 20000204; AU 2000027563 A AU 2000-27563

20000204
FDT AU 2000027563 A Based on WO 200046381
PRAI US 1999-131121P 19990427; US 1999-118634P 19990204; US 1999-122372P
19990302; US 1999-124905P 19990317
AB WO 200046381 A UPAB: 20001117
NOVELTY - A **recombinant** protein expression system (I) comprises a transgenic protozoan host cell resistant to paclitaxel containing a heterologous nucleic acid encoding a polypeptide, selectable by negative selection using paclitaxel.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an expression vector (II) comprising a 5' flanking region followed by a heterologous nucleic acid encoding a polypeptide comprising at least one targeting amino acid sequence encoded by a portion of an i-antigen-encoding nucleotide sequence from **Ichthyophthirius** multifiliis followed by a 3' flanking region, where at least a portion of each of the 5' flanking region and the 3' flanking region is complementary to an endogenous gene of an intended host, to allow integration into the endogenous gene by homologous **recombination**;

(2) a transgenic Tetrahymena thermophila comprising at least a portion of an I. multifiliis i-antigen protein;

(3) a transgenic cell (III) comprising a heterologous protein comprising at least one targeting amino acid sequence encoded by an i-antigen encoding nucleotide sequence from I. multifiliis;

(4) a method for preparing a polyclonal antibody;

(5) a method for detecting antibodies to an antigenic polypeptide comprising expressing the antigenic polypeptide on the surface of a transgenic protozoan host cell, exposing the host cell to an antibody and determining whether the swimming behavior of host cell is altered, where an alteration in the swimming behavior of the host cell is indicative of binding of the presence of antibodies to the antigenic polypeptide; and

(6) screening (IV) drugs for the ability to bind a polypeptide comprising expressing the polypeptide on the surface of a transgenic protozoan host cell, exposing the host cell to a drug and determining whether the swimming behavior of host cell is altered, where an alteration in the swimming behavior of the host cell is indicative of binding of the

drug to the polypeptide;

(7) a **vaccine** (V) comprising a transgenic non-pathogenic immunogenic protozoan comprising a surface-displayed antigenic polypeptide; and

(8) **recombinant** methods of producing a polypeptide.

ACTIVITY - Protozoacide.

MECHANISM OF ACTION - **Vaccine**.

Tetrahymena thermophila cells were transformed with the entire IAG48(G1) gene of I. multifiliis G1 which encodes the GPI anchored 48 kDa i-antigen or a truncated gene sequence. Transformants encoding the intact or C-terminal truncated i-antigen were grown in standard Tetrahymena growth medium. Groups of channel catfish were immunized intraperitonealy with T.thermophila transformants producing intact or truncated i-antigen. Fish were injected two times at a 30 day interval and challenged 21 days after the last immunization G5 **Ichthyophthirius**. The results showed that a greater degree of protection was elicited in immunized fish compared to controls. The antibody response of fish injected with live cells secreting **recombinant** i-antigen was two fold greater than the antibody response of fish immunized with Tetrahymena producing the membrane-bound intact i-antigen.

USE - The protein expression systems are useful for producing a polypeptide, comprising introducing (I) into a protozoan host cell that is resistant to paclitaxel, or a ciliated protozoan host cell to yield a transgenic protozoan host cell that is selectable by negative selection using paclitaxel and expressing the transgenic polypeptide in the transgenic protozoan host cell (claimed). The polypeptide is preferably an antigenic polypeptide and is expressed on the plasma membrane surface of the host cell and cleaved from the membrane surface of the transgenic host cell. Transgenic ciliated protozoa are useful as live **vaccines** for stimulating an immune response in a vertebrate. The transgenic protozoan host cells are useful for producing polyclonal antibodies (claimed). The cells are also useful for detecting antibodies to the antigenic polypeptide, by exposing host cells expressing the antigenic polypeptide on the surface to an antibody and determining alteration in the swimming behavior of the protozoan host cell, where swimming behavior of the cell is altered in the presence of the antibodies to the antigenic

polypeptide. The host cell is immobilized and exposed to the body fluid of
the patient suspected of being infected with the parasite (all claimed).
Tetrahymena expressing I.multifiliis i-antigen protein on their surface
are effective vehicles for **vaccination** of freshwater fish
against infection by I.multifiliis.
ADVANTAGE - The protein expression systems are suitable for large
scale and analytical scale production of **recombinant**
polypeptides and are particularly useful for expression of polypeptides
that are difficult to produce in conventional **recombinant**
protein expression systems.
Dwg.0/10

L38 ANSWER 6 OF 13 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD DUPLICATE
3
AN 2000-506071 [45] WPIDS
CR 2000-514962 [44]
DNC C2000-151959
TI Novel i-antigen polypeptides and polynucleotides from
Ichthyophthirius multifiliis, useful for prophylaxis and treatment
of **Ichthyophthirius** infection in fish.
DC B04 C06 D16
IN CLARK, T G; DICKERSON, H W; LIN, T
PA (CLAR-I) CLARK T G; (CORR) CORNELL RES FOUND INC; (DICK-I) DICKERSON H W;
(LINT-I) LIN T; (UYGE-N) UNIV GEORGIA RES FOUND INC
CYC 89
PI WO 2000046373 A1 20000810 (200045)* EN 144p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL
OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES
FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS
LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL
TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
AU 2000027561 A 20000825 (200059)
ADT WO 2000046373 A1 WO 2000-US2962 20000204; AU 2000027561 A AU 2000-27561
20000204
FDT AU 2000027561 A Based on WO 200046373
PRAI US 1999-131121P 19990427; US 1999-118634P 19990204; US 1999-122372P
19990302; US 1999-124905P 19990317
AB WO 200046373 A UPAB: 20001117
NOVELTY - An i-antigen polypeptide having a defined sequence of 442 or 468
amino acids (given in the specification) from a G1 or G5 isolate of
Ichthyophthirius multifiliis (or its fragment comprising
C-terminal or at least 1 terminal portion, its analog or an antigenic

fragment), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a nucleic acid molecule (II) comprising a polynucleotide fragment having a nucleotide that encodes (I);

(2) a nucleic acid molecule that is complementary to (II);

(3) a composition (III) for inducing an immune response in a fish comprising (I) or (II);

(4) a host cell transformed with (II);

(5) a fish comprising (II);

(6) transformed Tetrahymena comprising (II);

(7) an antibody capable of binding (I);

(8) identifying an **Ichthyophthirius** multifiliis serotype comprising providing a sample comprising a **Ichthyophthirius** multifiliis nucleic acid molecule having a nucleotide sequence encoding an i-antigen, adding to the sample at least 1 primer oligonucleotide having a sequence complementary to a unique region of the **Ichthyophthirius** multifiliis nucleotide sequence, and subjecting the sample to amplification conditions; and

(9) detecting (IV) **Ichthyophthirius** in an aquiculture, comprising obtaining a sample containing nucleic acid from an aqua culture fish or an aqua culture water, adding at least 1 primer oligonucleotide having a sequence complementary to a portion of a sequence of 1326 or 1404 base pairs (bp) (defined in the specification) to the nucleic acid sample, conducting a polymerase chain reaction (PCR) amplification with the sample.

ACTIVITY - Protozoacide.

MECHANISM OF ACTION - **Vaccine**.

Effect of I. multifiliis i-antigens to elicit protective immune response in channel catfish was studied. Fish were immunized by two intraperitoneal injections consisting of 10 mu g of purified 55 kD (kilodalton) i-antigen of the I. multifiliis G5 isolate in Freund's complete or incomplete adjuvant. As a positive control fish were **vaccinated** by two injections live, G5 parasites without adjuvant. All groups were challenged with infective G5 theronts 8 weeks after the last injection. Seventy-two percent of fish immunized with the i-antigen and 59.2% of fish immunized with live parasites survived the challenge while all of the negative control animals were dead.

USE - (III) is useful for prophylaxis, treatment or for controlling I. multifiliis infection in fish. Polynucleotide or protein **vaccine** comprising a portion of the amplified product encoding an

antigenic polypeptide obtained by (IV) is useful for treating or preventing I. multifiliis infection in fish (claimed). Dwg.0/21

L38 ANSWER 7 OF 13 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2000-13144 BIOTECHDS
TI **Recombinaint** expression systems for expressing heterogonous nucleic acids and producing **recombinant** protein such as Tetrahymena resistant to paclitaxel;
vector-mediated **Ichthyophthirius** multifiliis gene transfer and expression in Tetrahymena thermophilia for **vaccine** production
AU Gaertig J; Dickerson Jr H W; Clark T G
PA Univ.Georgia-Res.Found.
LO Athens, GA, USA.
PI WO 2000046381 10 Aug 2000
AI WO 2000-US2966 4 Feb 2000
PRAI US 990131121 27 Apr 1999; US 1999-118634 4 Feb 1999
DT Patent
LA English
OS WPI: 2000-514962 [46]
AB A **recombinant** protein expression system containing a transgenic protozoan host cell resistant to paclitaxel containing a heterologous nucleic cid encoding a protein, selectable by negative selection using paclitaxel. Also claimed are: an expression vector containing a 5' flanking region followed by a heterologous nucleic acid encoding a protein containing at least one targeting protein sequence encoded by a portion of an I-antigen nucleotide sequence from **Ichthyophthirius** multifiliis followed by a 3' flanking region, to allow integration into the endogenous gene by homologous **recombination**; a transgenic Tetrahymena thermophilia containing a portion of an I. multifiliis I-antigen protein; a transgenic cell; preparing polyclonal antibodies; detecting antibodies; screening drugs for the ability to bind a protein; a **vaccine** of a transgenic non-pathogenic immunogenic protozoan containing a surface-displayed antigenic protein; and producing a protein. The protein expression system is used for producing an antigenic protein for expression on the surface of a host cell for **vaccine** production. (83pp)

L38 ANSWER 8 OF 13 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2000-13109 BIOTECHDS
TI Novel i-antigen polypeptides and polynucleotides from **Ichthyophthirius** multifiliis, useful for prophylaxis and treatment of **Ichthyophthirius** infection in fish;
plasmid pProEX-1-mediated gene transfer and expression in Escherichia coli, Pichia pastoris or Tetrahymena sp. and antibody for

recombinant vaccine or nucleic acid **vaccine**
for gene therapy
AU Clark T G; Dickerson Jr H W; Lin T L
PA Univ.Georgia-Res.Found.; Cornell-Res.Found; Clark T G; Dickerson Jr H W;
Lin T L
LO Athens, GA, USA; Ithaca, NY, USA.
PI WO 2000046373 10 Aug 2000
AI WO 2000-US2962 4 Feb 2000
PRAI US 1999-131121 27 Apr 1999; US 1999-118634 4 Feb 1999
DT Patent
LA English
OS WPI: 2000-506071 [45]
AB An i-antigen protein having a defined 422 or 468 amino acid protein
sequence from a GI or G5 isolate of **Ichthyophthirius** multifiliis, is new. Also claimed are: a nucleic acid molecule containing a polynucleotide encoding the protein; a complementary nucleic
acid; a composition for inducing an immune response in a fish; a host
cell (e.g. Escherichia coli, Pichia pastoris or Tetrahymena sp.); a fish
containing the nucleic acid molecule; transformed Tetrahymena containing
the nucleic acid molecule; an antibody; identifying an I. multifiliis
nucleic acid encoding an i-antigen using a **DNA** primer; and detecting **Ichthyophthirius** sp. in an aquiculture using a **DNA** primer. Also disclosed are **vaccines** for preventing
diseases in fish caused by I. multifiliis and monoclonal or polyclonal
antibodies. The composition is useful for prophylaxis, treatment or for
controlling I. multifiliis infection in fish. A polynucleotide or
protein **vaccine** containing an antigenic protein obtained by the detection method is useful for treating or preventing I. multifiliis
infection in fish. In an example, plasmid pProEX-1 was used to transform
Escherichia coli. (144pp)

L38 ANSWER 9 OF 13 USPATFULL
AN 1998:82736 USPATFULL
TI **DNA**-based **vaccination** of fish
IN Davis, Heather L., Ottawa, Canada
PA Ottawa Civic Hospital Loeb Research, Ottawa, Canada (non-U.S. corporation)
PI US 5780448 19980714
AI US 1996-740805 19961104 (8)
PRAI US 1995-6290 19951107 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Mosher, Mary E.; Assistant Examiner: Salimi, Ali R.
LREP Fish & Richardson, P.C.
CLMN Number of Claims: 83
ECL Exemplary Claim: 1

DRWN No Drawings
LN.CNT 1309
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to methods of immunization of aquaculture species by introducing **DNA** expression systems into the aquaculture species. Such **DNA** expression systems preferably include **DNA** sequences encoding polypeptides of pathogens of species of aquaculture. The present invention also relates to methods of administration of **DNA** expression systems into aquaculture. Such methods include injection, spray, and immersion techniques. The methods of this invention are useful for prophylactic **vaccination** or therapeutic immunization of fin-fish, shellfish, or other aquatic animals against infectious diseases.

L38 ANSWER 10 OF 13 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
DUPLICATE 4
AN 1997-282877 [26] WPIDS
CR 1998-242678 [22]
DNC C1997-091054
TI **DNA vaccines** for aquatic sp., e.g. carp, trout and oysters - containing **DNA** sequence encoding immunogenic polypeptide.
DC B04 C06 D16
IN DAVIS, H L
PA (OTTA-N) OTTAWA CIVIC HOSPITAL LOEB RES INST; (OTTA-N) OTTAWA CIVIC HOSPITAL; (LOEB-N) LOEB RES INST OTTAWA CIVIC HOSPITAL; (LOEB-N) LOEB HEALTH RES INST AT OTTAWA HOSPITAL
CYC 9
PI EP 773295 A2 19970514 (199726)* EN 16p
R: DK FI FR GB SE
NO 9604713 A 19970509 (199729)
CA 2189831 A 19970508 (199736)
JP 09285291 A 19971104 (199803) 17p
US 5780448 A 19980714 (199835)
US 6180614 B1 20010130 (200108)
ADT EP 773295 A2 EP 1996-117859 19961107; NO 9604713 A NO 1996-4713 19961107; CA 2189831 A CA 1996-2189831 19961107; JP 09285291 A JP 1996-295565 19961107; US 5780448 A Provisional US 1995-6290P 19951107, US 1996-740805 19961104; US 6180614 B1 Provisional US 1995-6290P 19951107, Cont of US 1996-740805 19961104, US 1998-115423 19980714
FDT US 6180614 B1 Cont of US 5780448
PRAI US 1996-740805 19961104; US 1995-6290P 19951107; US 1998-115423 19980714
AB EP 773295 A UPAB: 20010207
DNA vaccine for aquaculture species comprises an expression control sequence capable of directing expression of at least 1 immunogenic polypeptide and a polypeptide-encoding **DNA** sequence

encoding at least 1 immunogenic polypeptide from the genome of a pathogen.

Also claimed are: (1) a pharmaceutical composition (I) comprising a **DNA vaccine** as above and a carrier; (2) a kit comprising (I) and at least 1 additional **DNA** expression vector comprising an expression control sequence capable of directing expression of at least 1 polypeptide and a polypeptide-encoding **DNA** sequence capable of facilitating an immune response for simultaneous, separate or sequential use in immunising an aquaculture species; (3) a kit comprising (I) and an adjuvant for separate or sequential use in immunising an aquaculture species; (4) a kit comprising (I) and a **recombinant** protein for separate or sequential use in immunising an aquaculture species to boost an immune response, and (5) a pharmaceutical composition for use in expression of a polypeptide in an aquaculture species, which comprises a **DNA** expression vector having an expression control sequence capable of directing the expression of at least 1 polypeptide and a polypeptide-encoding **DNA** sequence encoding at least 1 polypeptide.

USE - (I) is useful for immunising an aquaculture species by i.m. injection, i.p. injection, spray or immersion. The aquatic animal is selected from salmon, salmonid, carp, catfish, trout (including rainbow trout), yellowtail, sea-bream, sea-bass, eel, clams, lobster shrimp, crab and oysters. The composition is designed for immunisation against pathogens selected from viral haemorrhagic septicaemia virus, infectious haematopoietic necrosis virus, infectious pancreatic necrosis virus, spring viraemia virus of carp, channel catfish virus (Herpesvirus ictaluri), grass carp haemorrhagic virus, nodaviridae such as nervous necrosis virus or striped jack nervous necrosis virus, infectious salmon anaemia, Aeromonis salmonicida, Renibacterium salmoninarum, Yersinia sp., Pasteurella sp., Vibrosis sp., Edwardsiella sp., Streptococcus sp. and **Ichthyophthirius** sp. (all claimed).

Dwg.0/0

L38 ANSWER 11 OF 13 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 5
AN 1998:78040 BIOSIS
DN PREV199800078040
TI Protection of goldfish against **Ichthyophthirius** multifiliis by immunization with a **recombinant vaccine**.
AU He, Jiangyan; Yin, Zhan; Xu, Guoliang; Gond, Zhiyuan; Lam, Toong Jin; Sin,

Yoke Min (1)
CS (1) Sch. Biol. Sci., Natl. Univ. Singapore, 10 Kent Ridge Crescent,
Singapore 119260 Singapore
SO Aquaculture, (Dec. 1, 1997) Vol. 158, No. 1-2, pp. 1-10.
ISSN: 0044-8486.
DT Article
LA English
AB A 316 bp gene fragment containing a potential antigenic epitope of the 48
kDa immobilization antigen of **Ichthyophthirius** multifiliis (i-AgI) was assembled from six synthetic oligonucleotides and cloned into
a bacterial expression vector pGEX2T. The gene construct was introduced
into Escherichia coli and the glutathione S-transferase-iAgI (GST-iAgI)
fusion protein was successfully expressed. Antisera against GST-iAgI **fusion** protein from catfish showed a positive reaction with a tomites protein of about 48 kDa, suggesting that the **recombinant** protein contains an antigenic epitope of i-AgI. Naive goldfish that were immunized with purified GST-iAgI **fusion** protein were challenged with a lethal dose of infectious tomites of I.
multifiliis. The results showed that the average survival rate of the
immunized fish was 95% as compared to 55% for the control fish. All these
findings suggest that the **recombinant** GST-iAgI **fusion** protein can be used as a potential **vaccine** against the infection of I. multifiliis.

L38 ANSWER 12 OF 13 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1996-04421 BIOTECHDS
TI In vitro mutagenesis of an **Ichthyophthirius** surface antigen gene;
point mutation introduction into immobilization antigen, for subsequent expression and use as a **recombinant** **vaccine** in fish (conference abstract)
AU Dickerson H W; Clark T G
CS Univ.Georgia
LO Department of Medical Microbiology, College of Veterinary Medicine,
University of Georgia, Athens, GA, USA.
SO Conf.Res.Workers Anim.Dis.; (1995) 76 Meet., 163
CODEN: 9999G
Conference of Research Workers in Animal Diseases, 76th Annual Meeting,
Chicago, IL, 13-14 November, 1995.
DT Journal
LA English
AB The ciliate, **Ichthyophthirius** multifiliis (ICH), an important protozoan pathogen of freshwater fish. In the development of a subunit
vaccine against ICH, a class of putative protective surface antigens called immobilization antigens (i-antigens), has been identified. Sequence analysis of cDNA and genomic **DNA** encoding the i-antigens reveals that ICH has an anomalous genetic code such that

commonly recognized stop codons, TAA and TAG, code for the amino acid glutamine instead. Thus, expression of ICH genes using conventional bacterial systems results in the production of truncated proteins. 8 TAA and TAG stop codons in the first 419 nucleotides of the cDNA encoding the 48 kDa antigen of ICH strain G1 were altered using polymerase chain reaction-based mutagenesis and overlap extension, and they subsequently read as glutamine codons CAA and CAG, respectively, when cloned into bacterial cells for expression. A 429 bp product is currently being sequenced to confirm presence of the 8 point mutations. The mutated product will be cloned into the plasmid vector pFLAG for ultimate large scale expression of the **recombinant** protein antigen. (0 ref)

L38 ANSWER 13 OF 13 MEDLINE DUPLICATE 6
AN 94122710 MEDLINE
DN 94122710 PubMed ID: 8293000
TI Serotypic variation among isolates of **Ichthyophthirius** multifiliis based on immobilization.
AU Dickerson H W; Clark T G; Leff A A
CS Department of Medical Microbiology, College of Veterinary Medicine, University of Georgia, Athens 30602.
SO JOURNAL OF EUKARYOTIC MICROBIOLOGY, (1993 Nov-Dec) 40 (6) 816-20.
Journal code: BQT; 9306405. ISSN: 1066-5234.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199402
ED Entered STN: 19940314
Last Updated on STN: 20000327
Entered Medline: 19940225
AB Efforts have been made to determine whether surface antigens could be used as biochemical markers to define strain differences in the parasitic ciliate **Ichthyophthirius** multifiliis. In previous studies, a wild-type isolate designated G1 was found to have surface proteins analogous to the immobilization antigens of Paramecium and Tetrahymena; rabbit antiserum against this strain immobilizes homologous cells in vitro. It has now been shown for two additional **Ichthyophthirius** isolates (designated G1.1 and G2) that immobilization antigens are both present and serologically distinct. Proteins of similar size, which cross-react in Western blots with rabbit antisera against immobilization

antigens of the G1 strain, are nevertheless found in the G1.1 and G2 isolates. As shown by Southern blotting analysis, the G1.1 and G2 strains also contain genomic **DNA** sequences which hybridize with an immobilization antigen cDNA from G1 when probed under conditions of reduced stringency. The serotypic differences in immobilization between I. multifiliis isolates appear to be stable over time and provide a means of discriminating strains. In addition to providing a basis for comparative studies, the work described here has implications for the development of **vaccines** against this important fish parasite.